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SHORT COMMUNICATION

**Douglas M. Light · Alan L. Knight · Clive A. Henrick
Dayananda Rajapaska · Bill Lingren
Joseph C. Dickens · Katherine M. Reynolds
Ronald G. Buttery · Gloria Merrill
James Roitman · Bruce C. Campbell**

A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.)

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Abstract Ethyl (2*E*, 4*Z*)-2,4-decadienoate, a pear-derived volatile, is a species-specific, durable, and highly potent attractant to the codling moth (CM), *Cydia pomonella* (L.), a serious pest of walnuts, apples, and pears worldwide. This kairomone attracts both CM males and virgin and mated females. It is highly attractive to CM in both walnut and apple orchard contexts, but has shown limited effectiveness in a pear orchard context. Rubber septa lures loaded with ethyl (2*E*, 4*Z*)-2,4-decadienoate remained attractive for several months under field conditions. At the same low microgram load rates on septa, the combined gender capture of CM in kairomone-baited traps was similar to the capture rate of males in traps baited with codlemone, the major sex pheromone component. The particular attribute of attracting CM females renders this kairomone a novel tool for monitoring population flight and mating–ovipositional status, and potentially a major new weapon for directly controlling CM populations.

Introduction

For almost 30 years, codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) adults have been known to be attracted by the odor of apples (Wearing et al. 1973; Yan et al. 1999) and specifically to one of its volatile components, (*E*, *E*)-α-farnesene (Sutherland et al. 1974; Hern and Dorn 1999), based exclusively on laboratory bioassays. A key element limiting the development of (*E*, *E*)-α-farnesene as a semiochemical tool for CM management has been its instability and rapid chemical breakdown (Cavill and Coggiola 1971). Our objective was to identify other host-plant volatiles (HPVs) attractive to CM adults and to evaluate their potential utility in orchard pest management.

Gas chromatographic–mass spectrometric (GC–MS) analyses have shown that headspace trappings of complex odors of intact leaves of walnuts (Buttery et al. 1986; Campbell et al. 1999), pears (Miller et al. 1989; Scutareanu et al. 1997), and apples (Takabayashi et al. 1991), walnut husks (Buttery et al. 2000), and early-season immature apple or pear fruits are all dominated by a diverse array of mono-, sesqui-, and oxygenated-terpenoid HPVs. By contrast, HPVs of ripe apple and pear fruits are predominantly aliphatic esters, a few short chain-length aliphatic alcohols, and several sesquiterpenes [e.g., (*E*,*E*)-α-farnesene] (Carle et al. 1987; Nijssen et al. 1996). Since CM prefer pome fruits over walnuts (Barnes 1991), we hypothesized that HPVs unique to pome fruits might be readily perceived by and be more attractive to CM in a walnut orchard context.

D.M. Light (✉) · K.M. Reynolds · R.G. Buttery · G. Merrill
J. Roitman · B.C. Campbell
Western Regional Research Center, Agricultural Research Service, USDA, Albany, CA 94710, USA
e-mail: dlight@pw.usda.gov
Tel.: +1-510-5595831, Fax: +1-510-5595777

A.L. Knight
Yakima Agricultural Research Laboratory,
Agricultural Research Service, USDA, Wapato, WA 98951, USA

C.A. Henrick · D. Rajapaska · B. Lingren
Trécé Inc., Salinas, CA 93901, USA

J.C. Dickens
Vegetable Laboratory, Plant Science Institute,
Beltsville Agricultural Research Center,
Agricultural Research Service, USDA, Beltsville, MD 20705, USA

Materials and methods

The headspace-trapped volatiles (HSTV) of ripe Bartlett pears, apple varieties, and English walnut varieties were collected on Tenax® adsorbent traps and their odor compositions identified and compared following standard procedures (Buttery et al. 1986, 2000) using GC–MS with both DB-1 and DB-wax capillary columns and the methods of GC Kovat's Indices, MS spectra, and injections of authentic samples. Ninety-two HPVs unique to pome fruits were identified and chosen for behavioral screening in orchards. Twenty-three distinct blends were formulated in hexane, each blend composed of between two and nine pome fruit HPV constituents (each >95% purity), with a blend's constituents sharing a common carbon-chain length (from four to 15 carbons) and/or alcohol, aldehyde, or ester moiety. These 23 HPV blends, solvent controls (each at 10 mg/septum, gray halo butyl rubber; Trécé, Salinas, Calif.), and a commercial codlemone pheromone lure (Long Life® CM-L2®; Trécé) as a standard, were placed individually in monitoring traps (Pherocon® ICP or diamond-shaped II-B® traps; Trécé) and tested for relative attractiveness to CM. The individual synthetic constituents of the active blend(s) were subsequently field-tested. Traps were hung five trees apart in randomized blocks in California walnut and Washington apple orchards. Traps were checked and replaced at least weekly with moths being counted and sexed. Month-long tests were conducted during a 3 year period (1997–1999) in six orchards in both California and Washington.

Combined gas chromatographic–flame ionization detector coupled with electroantennographic detector (GC– EAD) recordings were performed on ten CM antennae of each sex in response to a 0.1 µg injection (0.1 µg/µl diluted in hexane) of HSTV of ripe Bartlett pears following established procedures (Dickens 1999). The GC (HP, model 5890) with a HP-5 capillary column (30 m) used a standard injector and oven temperature program (50° to 235°C max. at 15E C/min), flame-ionization detector, and 1:4 (FID:EAD) column splitter. The moths used were 2- to 4-day-old laboratory-reared CM (ARS – YARL, WA).

The most active kairomonal attractant candidate, ethyl (2*E*,4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD), was further field-tested in sensitivity and population monitoring studies. Pure samples (>98%; Trécé) of Et-*E*,*Z*-DD and CM synthetic sex pheromone, codlemone (*E*,*E*-8,10-dodecadien-1-ol), were serially diluted in methylene chloride and impregnated into gray rubber septa at decade loading rates, from 1 µg to 10 mg and 20 mg per septum. Randomized block field tests were conducted for 2-month periods in three walnut and apple orchards.

Comparisons of the identified kairomonal and pheromonal lures to monitor the seasonal emergence, population levels, and flight pattern of CM were conducted on populations endemic to walnut, apple, and pear orchards managed by conventional insecticide, organic, and mating disruption (MD) tactics. Diamond-shaped traps (II-B® traps; Trécé) baited with either Et-*E*,*Z*-DD (1–10 mg/septum) or commercial codlemone (CM-L2; Trécé) were hung 2–5 trees apart as competitively interactive pairs, with 2–15 replicate trap pairs distributed through 10–100-acre orchards. Monitoring tests were conducted and validated between 1998 and 2000 in an increasing number of orchards, from 3–25 walnut orchards (located near Davis, California) and 15–60 pome fruit orchards (located near Davis, California and Yakima, Washington). Traps were checked weekly (or more often) with moths counted and either removed from traps or traps replaced, while septa were replaced once per flight (about every 8–10 weeks). Moths were sexed and female moths were dissected to determine their mating status.

Results

Of the 23 blends tested, only the "C-10 ester blend" (a blend of methyl and ethyl esters of ten-carbon acids) attracted CM to traps. In walnut orchards, traps baited with the "C-10 ester blend" captured an average of 2.20±1.36 CM/trap per night (mean±SEM) with a sex ratio of 1 male:1.5 female, a combined-gender capture rate not significantly different (P=0.12, t-test) from the capture rate of males in synthetic sex pheromone-baited traps (3.15±0.98 CM males/trap per night). In all tests, traps baited with solvent controls did not capture CM. The principal attractive constituent in this synthetic blend was found to be ethyl (2*E*, 4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD) (1.45±0.33 and 1.38±0.30 CM/trap per night for walnut and apple orchards, respectively), which significantly (P<0.007, Duncan's multiple-range test) exceeded the attractiveness of other ester components in the blend, i.e., methyl (2*E*, 4*Z*)-2,4-decadienoate (0.17±0.5 and 0.31±0.13 CM/trap per night in walnut and apple, respectively), methyl decanoate (0.0 and 0.06±0.04 CM/trap per night respectively), or ethyl decanoate (0.0 CM/trap per night for both orchard types). Moreover, Et-*E*,*Z*-DD was as potent as pheromone as an attractant for CM in walnut orchards (pheromone:1.82±0.37%/trap per night; P=0.67, t-test) but was only half as potent in apple orchards in these particular tests conducted during the second-flight of CM in 1999 (pheromone: 2.83±1.11%/trap per night; P=0.04, t-test).

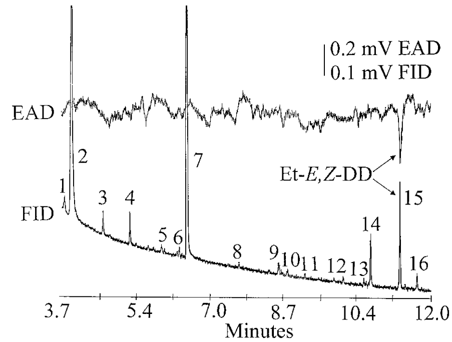

Fig. 1 Gas chromatographic–flame ionization detector coupled with electroantennographic detector (GC–EAD) recording from a male CM antenna (similar to female antennal recordings) in response to a 0.1 µg injection of headspace-trapped volatiles (HSTV) of ripe Bartlett pears (odor of 2.5 kg of fruit trapped for 24 h on Tenax® substrate). Identity and percentage composition (% of HSTV) of the numbered (1–16) GC-FID peaks are: *1*, ethyl butyrate (0.4%); *2*, butyl acetate (35.1%); *3*, hexan-1-ol (0.6%); *4*, pentyl acetate (1.1%); *5*, ethyl hexanoate (0.5%); *6*, (*Z*)-3-hexenyl acetate (0.1%); *7*, hexyl acetate (40.8%); *8*, heptyl acetate (0.2%); *9*, hexyl butyrate (0.6%) and ethyl octanoate (0.3%); *10*, octyl acetate (0.3%); *11*, ethyl (*E*)-2-octenoate (0.3%); *12*, methyl (*Z*)-4-decenoate (0.2%); *13*, ethyl (*E*)-4-decenoate (0.5%); *14*, methyl (2*E*, 4*Z*)-2,4-decadienoate (4.0%); *15*, Et-*E*,*Z*-DD (7.9%); and *16*, (*E*,*E*)-α-farnesene (0.9%)

GC–EAD analyses (Dickens 1999) of headspace trappings of ripe Bartlett pear odor revealed, for both male and female antennae, a definitive antennal chemoreception depolarization response (0.35 mV amplitude) elicited by the Et-*E*,*Z*-DD peak (7.9% of HSTV, approx. 7.9 ng stimulus, and retention time and identity confirmed by separate injection of authentic sample) (Fig. 1). No other obvious and temporally correlated EAD depolarization responses were observed to the other 15 FID detected peaks (Fig. 1).

In field dose–response sensitivity tests, Et-*E*,*Z*-DD exhibited comparable response threshold (a 10 µg dose per septum) and attractiveness for CM as codlemone over a range of substrate loading rates, from 0.1, 1.0, 10, and 20 mg per septum (Et-*E*,*Z*-DD: 1.15±0.40, 1.48±0.40, 3.10±0.42, and 1.47±0.44 CM/trap per night, vs pheromone: 2.29±0.47, 1.63±0.44, 0.93±0.31, and 0.86±0.31♂♂/trapper night). Based on this sensitivity, a

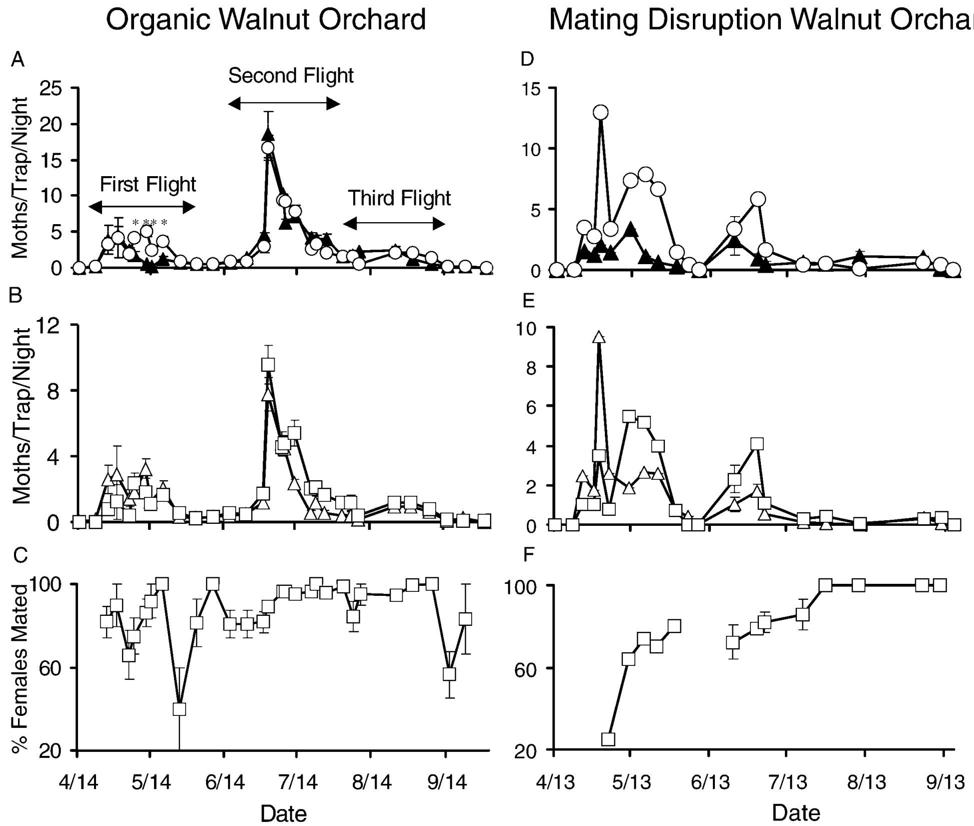

Fig. 2 Attributes of the ethyl (2*E*,4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD) kairomonal lure in monitoring 1999 seasonal and generation emergence, population levels, flight pattern, and mating status of codling moth (CM) populations endemic to an organically-managed walnut orchard (**A–C**) and a synthetic sex pheromone-permeated mating disruption (MD) walnut orchard (**D–F**). For the organic orchard example, traps baited with Et-*E*,*Z*-DD (1 mg/septum) and others with commercial sex pheromone (CM-L2; Trécé) were hung as pairs three trees apart, with 10–15 replicate pairs distributed through a 60-acre walnut orchard (Esparto, Calif.). **A** Comparison of Et-*E*,*Z*-DD and synthetic sex pheromone as a trap lures to time, scale, and delineate the three-flight seasonal activity of this walnut-infesting CM population. Combined capture of both sexes of CM in Et-*E*,*Z*-DD-baited traps (*open circles*) is significantly greater (P <0.05, paired t-test) than male CM capture in pheromone-baited traps (*solid triangles*) for a particular trap check date for only the 2 week period during the latter half of the first flight (*). **B** Seasonal flight activity of male (*open triangle*) and female (*open squares*) CM as resolved by trap monitoring with the Et-*E*,*Z*-DD lure. **C** Assessment of the mating frequency of captured females (*open squares*) and pattern. For the MD walnut orchard example, Et-*E*,*Z*-DD-baited (10 mg/septum) traps and standard sex pheromone-baited traps were hung as pairs in neighboring trees. Results were confirmed in additional MD-treated orchards, for 1999 in four walnut and six apple orchards and in 2000 in 19 walnut and 33 apple orchards. **D** Combined capture of both sexes of CM in Et-*E*,*Z*-DD-baited traps (*open circles*) in relation to males captured in pheromone-baited traps (*solid triangles*). **E** Seasonal flight activity of male (*open triangle*) and female (*open squares*) CM in a MD orchard as resolved by trap monitoring with the Et-*E*,*Z*-DD lure. **F** Assessment of percentage of mating occurring in the females (*open squares*) captured in Et-*E*,*Z*-DD-baited traps.

1 mg dose was used as the standard Et-*E*,*Z*-DD lure in orchards for periods of up to 2 months.

In all tests conducted, no endemic moth species other than CM nor “non-target” beneficial insects were caught in Et-*E*,*Z*-DD-baited traps. Only when rubber septa were impregnated with >20 mg of Et-*E*,*Z*-DD were other insect species captured, primarily stink bugs (Pentatomidae) and yellow jacket wasps (Vespidae).

Kairomone-baited traps detected, scaled, and tracked the seasonal CM flights well in both California walnut and Washington apple orchards (Fig. 2). For each flight, Et*E*,*Z*-DD-baited traps attracted more male than female CM in the initial phase of the first flight, then attracted more females than males in the second through third flights (Table 1 and Fig. 2B). Moreover, Et-*E*,*Z*-DD was generally equivalent to pheromone in attraction and capture efficacy of CM over the entire, three-flight season in walnut orchards (Table 1 and Fig. 2A). In Washington apple orchards, the capture rate of Et-*E*,*Z*-DD-baited traps was equivalent to that of pheromone-baited traps for the first flight period of CM (Table 1). However, differences over the last two seasons (1999 and 2000) in trap capture efficacy of the two lures in apple orchards were observed during the second flight period of CM. Kairomone capture rates diminished relative to continued strong pheromone attraction in apple orchards during the second flight of the 1999 season, while the opposite trend was observed in the 2000 season with kairomone trap capture significantly (P=0.04, t-test) exceeding that of pheromone-baited traps (Table 1). In contrast, this kairomone appears not to be a strong or effective lure in pear orchards (Table 1).

Table 1 Capture rates of codling moths (moths/trap per night) in traps baited with ethyl (2*E*,4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD) in comparison to traps baited with codlemone (synthetic sex pheromone) over the seasonal flight periods in three host species orchards managed under conventional (*conv*) and mating disruption (*MD*) practices in 1999 and 2000. Means (±SEM) are averages of the average capture rates of CM/trap per night for 3–13 individual orchards, with the average for each orchard based on 2–15 replicated treatment traps and weekly to more frequent (2-day) trap check intervals. Gender capture rates to the kairomone and total capture with kairomone lure vs male capture with pheromone lure marked with * or ** were significantly different from each other within a row, paired *t*-tests

Year and host orchards		Mean capture (±SEM) CM/trap/night			
		Et-*E*,*Z*-DD kairomone			Pheromone Males
		Females	Males	Total	
		1st flight			
1999:	Walnut, conv (7 orchards)	2.52±0.76	3.15±0.48	5.67±1.17	5.97±1.83
2000:	Walnut, conv (12 orchards)	1.24±0.21	1.46±0.23	2.81±0.37	3.62±0.49
	Walnut, MD (13 orchards)	1.03±0.18	0.76±0.17	1.79±0.31	0.14±0.09
1999:	Pear, conv (3 orchards)	0.32±0.04	0.49±0.20	0.81±0.24**	3.35±1.36**
	Apple, conv (3 orchards)	1.93±0.51	2.45±0.69	4.38±0.95	3.95±0.73
2000:	Apple, conv (6 orchards)	0.24±0.03	0.63±0.06	0.87±0.09	0.90±0.19
	Apple, MD (6 orchards)	0.09±0.03	0.24±0.08	0.33±0.09	0.41±0.11
		2nd flight			
1999:	Walnut, conv (7 orchards)	2.77±0.54	2.03±0.57	4.79±1.06	3.87±0.86
2000:	Walnut, conv (12 orchards)	1.86±0.49	0.93±0.17	2.79±0.56	3.27±0.51
	Walnut, MD (13 orchards)	1.34±0.32	0.22±0.05	1.56±0.36	0.12±0.05
1999:	Pear, conv (3 orchards)	0.02±0.02*	0.32±0.17*	0.35±0.17**	3.54±0.59**
	Apple, conv (3 orchards)	0.26±0.12	0.21±0.09	0.47±0.04**	3.20±1.24**
2000:	Apple, conv (6 orchards)	0.30±0.06	0.50±0.11	0.80±0.17*	0.47±0.11*
	Apple, MD (6 orchards)	0.29±0.07	0.38±0.10	0.67±0.17*	0.28±0.13*
		3rd flight			
1999:	Walnut, conv (7 orchards)	1.34±0.46	0.86±0.41	2.19±0.83	2.30±0.89
2000:	Walnut, conv (12 orchards)	0.91±0.23	0.63±0.16	1.55±0.38	1.61±0.31
	Walnut, MD (13 orchards)	0.49±0.10*	0.10±0.03*	0.59±0.11*	0.16±0.10*

*$P<0.05$, **$P<0.01$.

The Et-*E*,*Z*-DD kairomonal lure attracted both virgin and mated females in all tests conducted. Examination of captured females showed that mating fluctuated but generally increased as the first flight progressed, decreased between flights, increased as the next flight initiated, and reached a sustained maximum with most females mated (approx. >95%) once the second and third flights reached their peaks (Fig. 2C). Similar flight trends were observed using Et-*E*,*Z*-DD-baited monitoring traps in a walnut orchard managed solely by MD (Fig. 2E, F). The distinct feature observed in MD orchards was that Et-*E*,*Z*-DD–baited traps captured greater numbers of moths than codlemone-baited traps (Fig. 2D, Table 1).

Discussion

Ethyl (2*E*, 4*Z*)-2,4-decadienoate has been found to be a new and effective attractant for both male and female CM. Moreover, Et-*E*,*Z*-DD evokes a highly species-specific attraction of CM. The attractive behavior expressed in response to this pear-derived volatile demonstrates the high sensitivity and responsiveness of CM to this kairomone. GC–EAD recordings reveal a specific chemoreceptive affinity and sensitivity of CM to Et-*E*,*Z*-DD. The only definitive and large EAG depolarization response of both male and female antennae was elicited by the Et-*E*,*Z*-DD peak and not by any other odor constituent of ripe Bartlett pears.

Et-*E*,*Z*-DD is a major and key odorant of ripe pear fruits (commonly named the "pear ester"), accounting for up to 10% of total headspace volatiles emitted (Fig. 1) and even higher levels in steam distillates (Jennings et al. 1964). However, this ester is not detected in immature pome fruit or pear-leaf volatiles (Miller et al. 1989; Scutareanu et al. 1997). Though not detected in our analyses, Et-*E*,*Z*-DD has also been reported as a minor constituent in the aromas of ripe, picked apples (Berger et al. 1984) and quinces (Shimizu and Yoshihara 1977). Et-*E*,*Z*-DD has not been identified as an insect pheromone (Mayer and McLaughlin 1991), although the corresponding methyl ester, methyl (2*E*, 4*Z*)-2,4-decadienoate, is a key component of the pheromone of a number of stink bug species (Pentatomidae: *Euschistus*) (Aldrich et al. 1991).

The lack of strong or effective CM attraction to this kairomone in pear orchards might be based on its origin. This pear-odorant lure is chemically very different from terpenoid-based odors of walnut and early-season apple orchards (Buttery et al. 1986, 2000; Takabayashi et al.

1991). Reduced moth capture activity of Et-*E*,*Z*-DD in pear orchards and during the second half of the season in apple orchards might be due to olfactory 'masking' or competitive effects of the same ester or similar esters and other natural semiochemicals released as these pome fruits mature (Heinz et al. 1965; Shiota 1990; Yahia et al. 1990; Mattheis et al. 1991) and/or incur damage (Boevé et al. 1996; Landolt et al. 2000).

Although pheromone traps are the current standard means of monitoring pest flight patterns, the information derived pertains only to male moth activity. The use of Et-*E*,*Z*-DD as an attractant alternately enables resolution of moth emergence and flight patterns (including onset, peak, intensity, and duration) for both sexes in both conventional and especially MD-managed orchards. Moreover, this kairomone provides a means for the assessment of the timing of female emergence, flight, and mating activities (Fig. 2), which is the crucial and pertinent information for decision-making and effective and judicious timing of insecticidal control measures. The potential use of this kairomonal attractant for direct control measures which target both male and female moths or larvae (Knight and Light 2001) holds great promise for CM management.

The composite of our studies shows Et-*E*,*Z*-DD is a strong kairomonal attractant for both sexes of CM, exhibiting a similar degree of species-specificity, chemical specificity, and potency as CM sex pheromone for male attraction. The key distinction between these CM lures is that the kairomone attracts both sexes, including mated and unmated females. The high degree of species specificity and potency (1 mg doses being effective for months) creates a potential practical use for Et-*E*,*Z*-DD that is unique among host-plant-derived insect kairomones. In contrast, most agriculturally utilized plant-derived kairomonal lures (Waage and Hedin 1985; Metcalf 1987) require a blend of constituents and must be formulated with substantially greater amounts of active ingredient(s) (hundreds of micrograms to many grams) to elicit field attraction for much shorter periods, usually only weeks, e.g., adult apple maggot (Fein et al. 1982; Zhang et al. 1999), or male *Bacterocera* fruit flies, adult *Diabrotica* corn root worms, and Japanese beetles (Metcalf 1987).

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